Docket No. 46745 (1758)

IN THE UNITED STATES PATENT AND TRADEMARK_OFFICE

APPLICANT:

J. Weidanz, et al.

SERIAL NO .:

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EXAMINER: M. Lubet

FILED:

March 7,

GROUP:

1644

FOR:

FUSION PROTEINS COMPRISING BACTERIOPHAGE COAT

PROTEIN AND A SINGLE-CHAIN T CELL RECEPTOR

THE HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS WASHINGTON, DC 20231

SIR:

DECLARATION PURSUANT TO 37 CFR 1.131

The undersigned declare as follows:

- 1. We are co-inventors of the above-identified application (hereafter the "subject application"). Jon A. Weidanz and Hing Wong are Senior Scientists with Sunol Molecular Corporation, Miramar Florida. Hing Wong is currently President and CEO of that corporation.
- 2. As we understand it, the subject application discloses and claims, among other things, fusion proteins in which bacteriophage coat protein is covalently linked (ie. fused) to a soluble single-chain T-cell receptor. As one example, the subject application discloses and claims, a single-chain T-cell receptor linked to a bacteriophage VIII coat protein ("single chain fusion protein").
- 3. We have reviewed the Patent Office Action ("Office Action") dated June 23, 1999 issued in connection with the subject application. As we understand the Office Action, the patent Examiner rejected claims 1, 2, 4, 7-8, 14, 18, 19, 20, 61 and 67 as obvious in view of WO 96/18105 ("Strominger") taken with other references

cited in the Office Action. We understand that Strominger is a PCT application having an issue date of 13 June 1996.

- 4. We have read Strominger. As we understand it, Strominger discloses a specific single-chain T-cell receptor that binds to an MHC peptide ligand.
- 5. The invention described and claimed in the subject application was conceived and reduced to practice in the United States prior to June 1996.
- 6. For example, well before June 1996, we recognized that it was possible to make a recombinant T-cell receptor (TCR) protein in which a V-alpha chain was fused to a V-beta C-beta chain by a peptide linker sequence. It was also recognized before the June 1996 date that a bacteriophage coat protein could be fused to the V-beta C-beta chain to produce the single-chain fusion protein. As understood, use of the bacteriophage gene VIII coat protein could substantially improve features of the fusion protein. As disclosed throughout the subject application, that recognition helped us make and use the single-chain fusion protein.
- 7. The claimed single-chain fusion protein was made in the United States well before Strominger's June 1996 publication date.
- 8. For example, conception of the single-chain fusion protein was accomplished well before Strominger's publication date. Attached as **Exhibit 1** is a true and accurate copy, with dates deleted, of notes made by one of us before June 1996. For example, see pages 1 and 2 of the notes in which is shown, among other things, drawings of single-chain fusion protein. Also indicated in the notes, among other things, are steps for making vector encoding peptide linker sequence fused to the bacteriophage gene VIII protein. The vector is referred to as pKC27. Construction and use of the pKC27 vector is further exemplified by the subject application. For

instance, see Example 2 and particularly part E which discloses work performed well before the June 1996 date. See also Figure 2 of the application (outlining production of pKC27 and other vectors).

- 9. One of us prepared sequence encoding TCR V-alpha chain well before Strominger's June 1996 publication date. Attached as **Exhibit 2** is a true and accurate copy of notes, with dates deleted, prepared by one of us well before that date. The notes show, among other things, successful production of sequence encoding the V-alpha chain. These manipulations were performed well before the June 1996 date and are described throughout the subject application, for instance, see Example 1.
- fused sequence encoding the V-alpha chain into the pKC27 vector. That manipulation led to production of pKC42 vector. The pKC42 vector particularly encodes sequence in which the V-alpha chain is fused to the peptide linker. That linker is fused to the bacteriophage gene VIII protein. Attached as **Exhibit 3** is a true and accurate copy of notes made by one of us, with dates deleted, describing steps taken to make the pKC42 vector among other things. These steps were undertaken well before the June 1996 date and are further exemplified in the subject application. For instance, see Example 2 and particularly section E. See also Figure 2 of the subject application (outlining production of pKC42 from the pKC27 vector).
- 11. We made vectors that encode the TCR V-beta C-beta chain well before Strominger's June 1996 publication date. Attached as **Exhibit 4** is a true and accurate copy of notes made by one of us, with dates deleted, that shows, among other things, steps taken to make pKC30 vector. These manipulations were conducted well before the June 1996 date and are further exemplified in the subject application. See Figure 2, for example.

- 12. In experiments performed well before Strominger's June 1996 publication date, we made vectors that encode the V-beta C-beta chain fused to the bacteriophage gene VIII protein. Attached as **Exhibit 5** is a true and accurate copy of a note made by one of us, with dates deleted, which shows, among other things, manipulation of a vector called pKC34.3. The pKC34.3 vector is a specific isolate of pKC34 and it is further exemplified in Figure 2 of the subject application (see the step for making pKC44 from the pKC42). The note particularly describes treatment of pKC34.3 with restriction endonucleases to isolate a fragment encoding the V-beta C-beta chain fusion protein. Steps taken to make the pKC34.3 vector were performed well before the June 1996 date.
- sequence encoding the V-beta C-beta bacteriophage gene VIII fusion protein into the pKC42 vector. Attached as **Exhibit 6** is a true and accurate copy of notes made by one of us, with dates deleted, showing, among other things, production of the pKC44 vector (encodes single-chain fusion protein). In particular, pages 1-5 of the notes show manipulation of specific pKC42 vectors (42.1, 42.2, and 42.3) and use of those vectors as recipients of sequence encoding the V-beta C-beta bacteriophage gene VIII construct. These results are further exemplified by the subject application. For instance, see Example 2 and particularly section E. See also Figure 2 of the application (showing production of pKC44 from pKC42).
- 14. We made the single-chain fusion protein in the United States well before the June 1996 publication date of Strominger.
- 15. For example, attached as **Exhibit 7** is a true and accurate copy of notes made by one of us, with dates deleted, that shows, among other things, manufacture of the single-chain fusion protein well before Strominger's June 1996 date. In particular, pages 1-4 of the notes show expression of the single-chain fusion protein

encoded by pKC44 as evidenced by a Western Blot. Pages 5 and 6 of the notes show purification of that protein using an immuno-affinity column. These manipulations are further exemplified by the subject application. For instance, see Examples 4, 5, 6 and Figures 7-13 which disclose work performed well before June 1996.

16. We hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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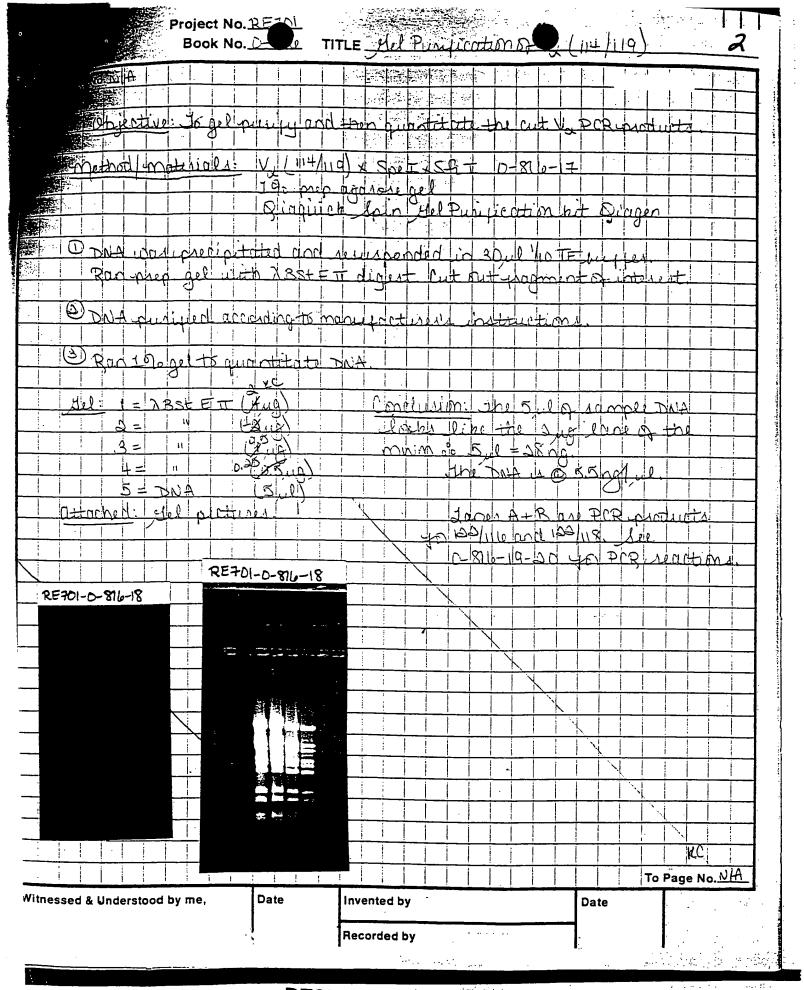
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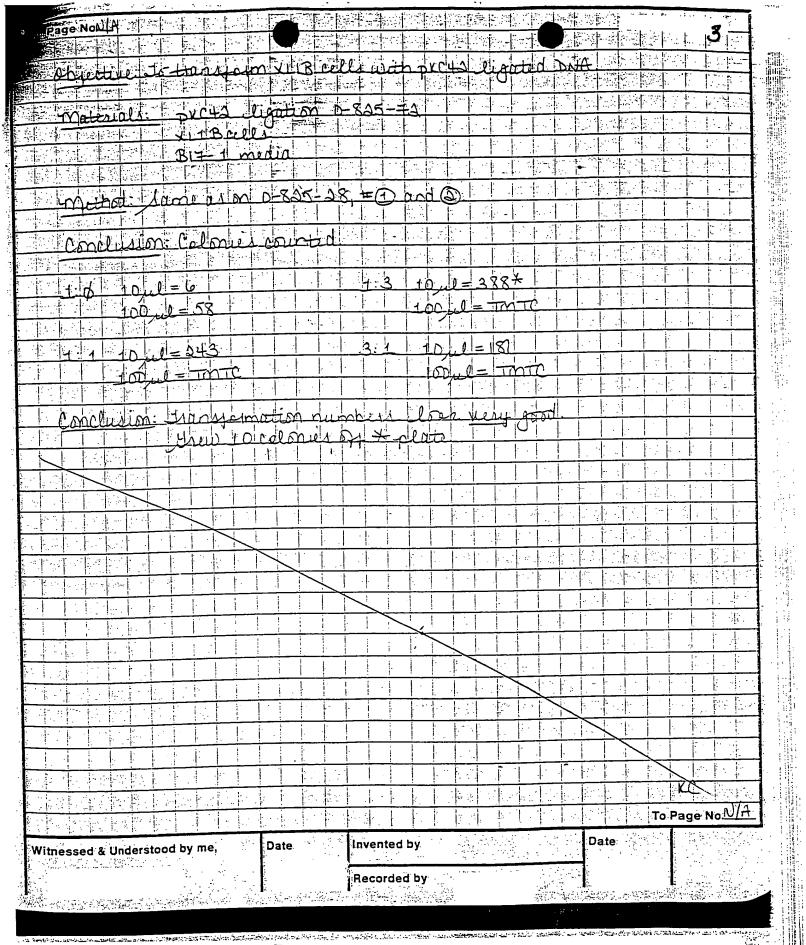
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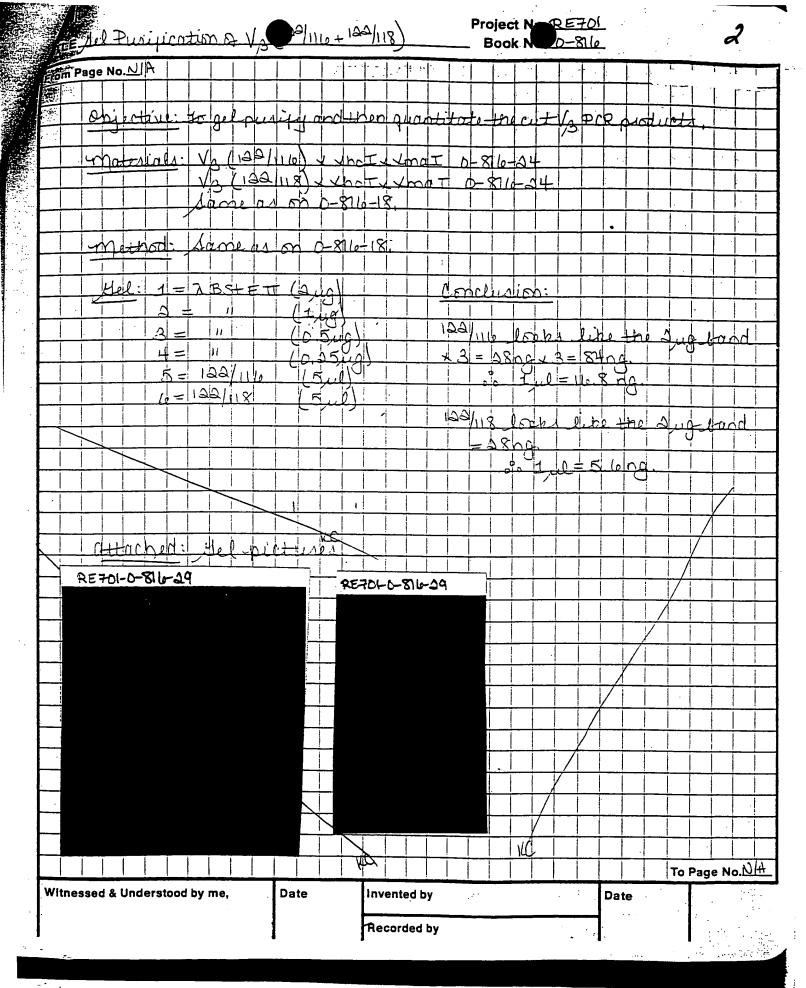
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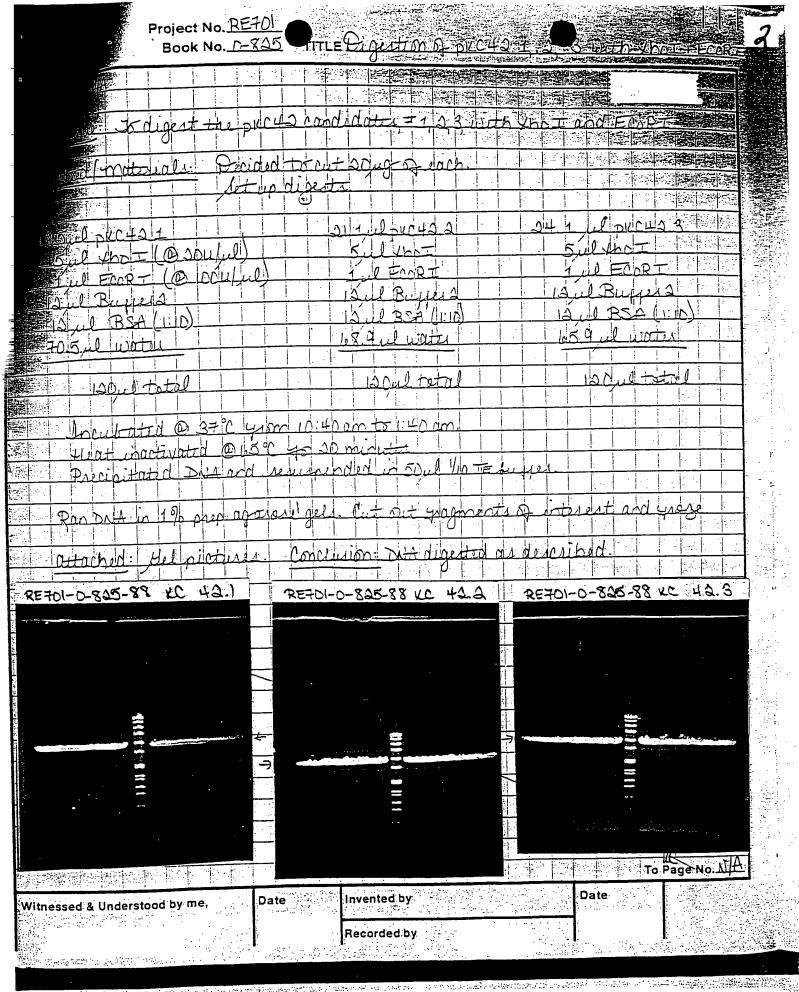
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Objective: Younduce the Fab constructs con-
taining the DOILID cell cline's TCR (pxc3435,
40, and 41) and the SC constructs (pKC43 and
44). PICI8 is a positive control to the Bchain.
materials:
cells all adapted to high phosphate media
high phosphate media clow-phosphate media
protein extraction lupper (R77) urea
media @ 30°C with shaking-5mleach.
media @ 30°C with shaking-5ml each.
attached: Basic induction spectocal.
INDUCTION OF THE phoA PROMOTER SYSTEM:
Small scale:
Grow overnight culture in high phos. medium (B5) @ 30°C5 mls is enoug
(2) Harvest Zmls into each of two tubes
Pellet cells (gently)wash one pellet with high phos. medium, and the other
with low phos. medium (B8)
Repeat once
Resuspend the appropriate pellet with 2 mls high phos. medium, and the otl
with 2mls low phos. medium. only to some construits:
Inoculate the resuspended cells into 100mls of the appropriate medium
Induction is carried out with growth @ 30°C for 4+ hours, overnight.
- NOTE: Both B5 and B8 already contain ampicillin!
Inductions were carried out 40, 5 hours of over-
night as neted:
$5.hu. \rightarrow 34, 35, 40, 44, 43, 44, 18$
5 hr. non-induced -> 44
ON-> 34,35,40,41,43,44,18
ON non-induced > 44,18 BEST AVAILABLE COPY

	Induction of Fab and Single Chan Constructs	2				
1 1000	3 DD 600 s were measured:					
	34-5 (0.04 x 2 = 0.08) -> use all 50ml = 10 0Ds					
	35-5 (0.07 x 2 = 0.14) "					
	40-5 (015x5=0.75) 13.3 ml "					
	41-5/0.1 x5= 0.5) 20.0 ml "					
	43-5 (0.03 x neat = 0.03) all "					
	44-5 (0.1×5=0.5) 20.0ml "	<u></u> -				
	18-5 (0.12×5=0.6) 16.7 ml "					
	44N-5 (0.055×10=0.55) 182ml "					
2 ・	34-0N (0.09×10=09) 11.1 ml "					
	35-00 (0.21 × 10 = 2.1) 4.8 ml "					
	40-00 (0.09 ×10 = 0.9) 11.1ml "					
	41-ON (D.11 XID=1.1) 9.1 ml "					
	43-0N (0.05×10=0.5) 20.0 ml "					
	44-DN (D. 26×1D=26) 3.8 ml	e e sero al gracione e s				
	18-0N (D.1×10=1.0) 10.0ml "	auri — manar filtring an f				
	44N-DN (0.4×10=4.0) 2.5 ml "					
	18N-ON (0.37×10=3.7) 2.7ml "					
U						
E \$7	4 Cells (volumes listed 40 10 ODs) were spundown)				
	Pellets resuspended in Indepotein certification					
AVAII A	buffer. Sonicated 2 minutes using cup sonicator.					
5	Spun 5 minutes @ maximum speed. Supe =					
8 9	soluble yaction. Resuspended pellets in Inl					
	A same buffer + 8 m usea. Rocked 12 hour @s	DD-CY_				
COPY	temperature and spun 5 minutes @ maximum					
	speed. Supe. = insoluble fraction.					

Stopertime: Jo look @ E cali lysotes for the of either Fab ITER of single chain ITER.

2x cracking Juffer w/same Materials: lysates prepared on p. 4-5 12.590 SDS-PAGE gels membrane slatts

Bailed 5 minutes. Run out on 12.5 he geld. metted Odysates were diluted 1:3 in Exeracking hupper (10 ml. lydate + 10 ml hupper, per larre)

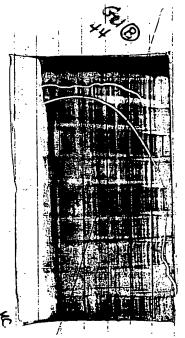
@ Hansperied to membrane for 15 minutes @ 250-300 manages

(3) Bloched 1 how in flatto diluted 1:10 in 1x PBS. @ 1:1.5K_(26jul 1:50 in 1x PBS. Wanted Weished 1x. Presed you I how with a - 4/3 TCR - HRP substrate 40 1 minute. Exposed film. [39 ml huger) in flotto dillited 3x, Exposed to ECL HRP

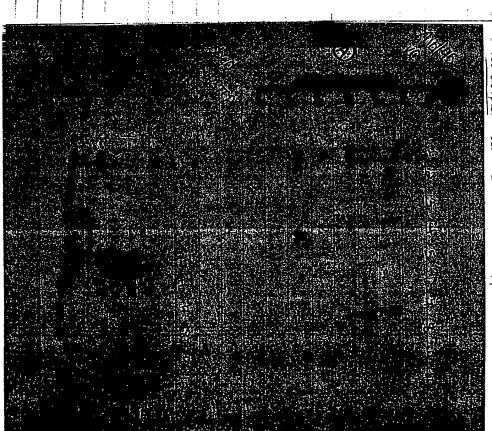
Uttached: Comassie stained-gel

Western Blat + Comassee the of Lyantes

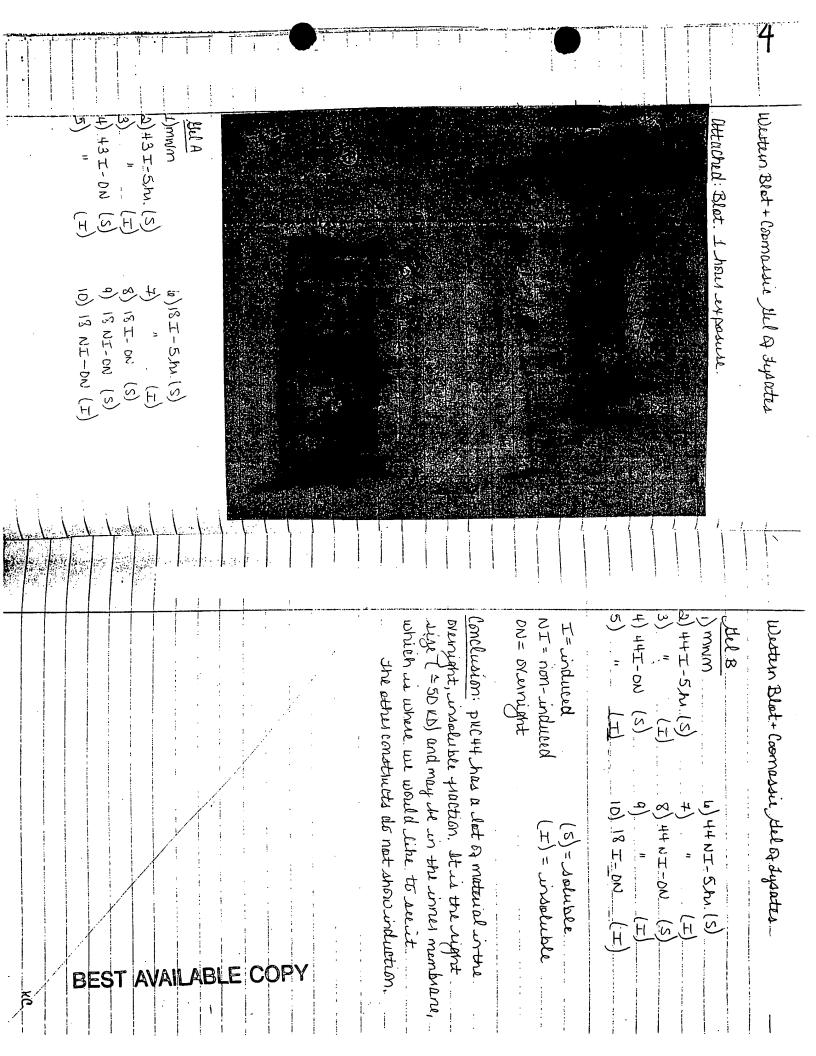
attached: Compasse there stained get



attached: Blat. 10 missite exposerse



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									5
Depun To soluble quartion, added CNBrz-4V58.2	(3) Let solubilize @4°C with shaking / wocking for	Resuspended cell-posts in hyper. Passed through yheren press traile	15 the 150 ml of Juffer, added 3 tallets of "Complete" (protease inhibiter from Boschninger marricheim). Let disselve completely.	130g 2000ml = x x=516ml x30.7 co)ml = 105 6 00)ml = 15,841.2 co = 105 6 00)ml	00 = 30.7 ml ob = 30.7 ml ob decided to manaperd paste in 150ml	Bruided to resuspend poste @ = 10000 ml in tuffer. (Buffer = 50mm true, 150mm Nace, 0.5% NP40)	method: O started with ss. 36 g of furments paste you d-supschen. Jet=0-788:91, furments+1, 9/13/95,	abjective: Want to try printy column.	Punisication of SCTCR-vaing-a-V58.2
BEST AVAILABLE COPY	Conclusion: scTCR prep. (let # 5) prepared.	membranes and washed Sx. Rivard membrane. Chucked pH- ok. Final volume = 340 rd in heped	19 Popled sample from your trues to two trues and runsed membranes washed sx	(aso)+ 0.1% North and spin as above. Washed 3x.	neutralized each fraction with 200 ml 200 times. PHS.D. Defit fraction = 2 among your microcon-100 concer-	Eluted two fractions: fraction # 1 = Inlot 0.1 m glycine + 0.1% NPHO fraction # 3 = Inlot ", pH 3.0	Whoseled resin together in I take and washed ix with more supper Janagened resin to column and washed ix with more supper	© 800 spons you & minutes to pull down resionatived auper as "ylow-thu".	Puniqueation of SCTCR

commassie Hel of Furified SCTCR Lat#5

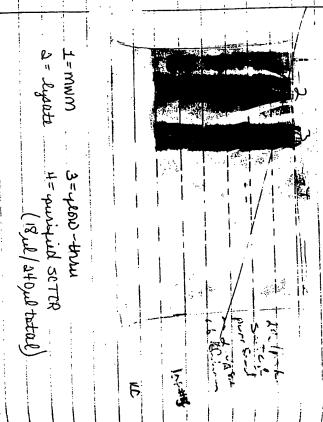
chipective: For look @ the purified SCTCR.

This lot was purified with the d-V38.2 Ab.

method: I same as on page 44 but did not

men a western slot.

4+12ched Comassic stained gel

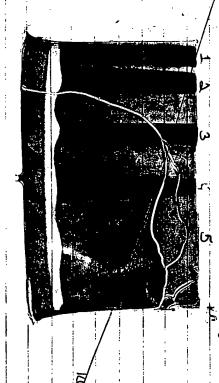


Western Blat + Coornasie Helof SCTCR (lat # 6)

This let was purified with toth the x-YS. 2 and x-43TCR Ab columns combined.

method: Same as on page 74.

attached Comassie stained gel



1= mwm

4= waah#1

3= 46w-4hru

Conclusion: Can see puriqued SCTER Land!

5 - puriqued SCTCR_

Conclusion: Purified SCTCR Closks good!

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